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A colorimetric chemosensor for the sequential detection of copper(II) and cysteine

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ABSTRACT

A simple and easy-to-make colorimetric chemosensor **1** for the sequential detection of Cu^{2+} and cysteine was developed by combination of hydroxynaphthalene-2-carbaldehyde and diaminomaleonitrile. This sensor **1** exhibited an obvious color change from pale yellow to orange in the presence of Cu^{2+} in aqueous solution. Also, the resulting **1**-Cu^{2+} complex sensed cysteine through naked-eye, showing recovery from **1**-Cu^{2+} to **1**, over other sulfur-containing amino acid and peptide. Moreover, the sensing ability of **1** for Cu^{2+} was supported by theoretical calculations.

1. Introduction

The design and development of selective optical chemosensors are an important task for scientific researchers [1–3]. Especially, transition metal ions have received an increasing concern due to their importance in many biological and environmental processes [4]. In particular, Cu^{2+} is the third most abundant essential trace element in the human body. Various redox processes, enzyme functions, and pigments are dependent on copper ions as cofactors [5–7]. However, excess concentration of Cu^{2+} in the neuronal cytoplasm can cause Wilson and Menkes disease [8,9]. The World Health Organization (WHO) has recommended the maximum limit of copper in drinking water to be at 2 ppm (30 μM) [10,11]. For these reasons, much effort has been devoted to the design of various chemosensors specific for Cu^{2+} detection [12–18].

Cysteine (Cys), one of the intracellular thiol-containing amino acids, plays critical roles in biological systems. It is a functional compound of many proteins and enzymes [19,20]. However, abnormal levels of biological Cys can lead to several health problems. For example, Cys deficiency can cause slow growth, hair depigmentation, edema, lethargy, liver damage, muscle and fat loss, skin lesions, and weakness [21–24]. Hence, the determination of trace amounts of Cys is still highly demanded. Although significant researches on the chemosensor of fluorescence changes for Cys have been reported, the chemosensors based on color changes for Cys in aqueous solution remain very rare [25–30].

Recently, Schiff base derivatives of diaminomaleonitrile that contain coumarin-3-yl, naphthal-1-yl, and pyren-3-yl units were used as chemosensors for the colorimetric and fluorometric determination of Cu^{2+} ions, respectively. However, these probes show a lack of selectivity in the presence of Fe^{3+} and Fe^{2+} or sensing ability in aqueous solvent [31–33]. Therefore, these results led us to develop a new effective chemosensor based on diaminomaleonitrile.

Herein, we report a new diaminomaleonitrile-based chemosensor **1** as a selective multifunctional colorimetric recognition of Cu^{2+} and Cys. The chemosensor **1** exhibited a color change from yellow to orange upon binding to Cu^{2+} in aqueous solution. Moreover, it could detect Cys via sequential recognition showing recovery color and absorption band of the chemosensor **1**.

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2. Experimental

2.1. Materials and instrumentation

All the solvents and reagents (analytical and spectroscopic grade) were purchased from Sigma–Aldrich. ^{1}H and ^{13}C NMR spectra were recorded on a Varian 400 MHz and 100 MHz spectrometer and chemical shifts are recorded in ppm. Electro spray ionization mass spectra (ESI-MS) were collected on a Thermo Finnigan (San Jose, CA, USA) LCQ$_{TM}$ Advantage MAX quadrupole ion trap instrument by infusing samples directly into the source using a manual method. Spray voltage was set at 4.2 kV, and the capillary temperature was at 80 °C. Absorption spectra were recorded at room temperature using a Perkin Elmer model Lambda 2S UV/Vis spectrometer. Elemental analysis for carbon, nitrogen, and hydrogen was carried out using a Flash EA 1112 elemental analyzer (thermo) at the Organic Chemistry Research Center of Sogang University, Korea.

2.2. Synthesis of receptor **1**

An ethanolic solution of hydroxynaphthalene-2-carbaldehyde (0.36 g, 2 mmol) was added to diaminomaleonitrile (0.11 g, 1 mmol) in absolute ethanol (3 mL). The reaction solution was stirred for 30 min at room temperature and the solvent was removed in vacuo. The crude product was filtered and washed using ethanol and ether. Yield 0.16 g (48%); ^{1}H NMR (400 MHz DMSO-d_6, ppm): δ 12.02 (s, 1H), 9.25 (s, 1H), 8.60 (d, J = 8 Hz, 1H), 8.01 (d, J = 8 Hz, 1H), 7.91 (m, 3H), 7.63 (t, J = 8 Hz, 1H), 7.43 (t, J = 8 Hz, 1H), 7.24 (d, J = 12 Hz, 1H); ^{13}C NMR (100 MHz DMSO-d_6, ppm): 159.85, 154.94, 135.33, 131.73, 129.00, 128.68, 127.94, 125.21, 124.00, 122.13, 118.55, 114.74, 114.20, 110.32, 103.94, 56.07, 18.60. ESI-MS m/z (M− H$^+$): calcd, 261.08; found, 261.23. Anal. Calc. for $C_{15}H_{10}N_4O$: C, 68.69; H, 3.84; N, 21.36; Found: C, 68.35; H, 3.94; N, 21.52.

2.3. UV–vis titration

For Cu^{2+}, **1** (7.8 mg, 0.003 mmol) was dissolved in dimethylsulfoxide (DMSO, 1 mL) and 10 μL of this solution (3 mM) were diluted with 2.99 mL of 10 mM bis-tris buffer/DMSO (7/3, v/v) to make the final concentration of 10 μM. $Cu(NO_3)_2\cdot 2.5H_2O$ (2.4 mg, 0.001 mmol) was dissolved in 10 mM bis-tris (1 mL) and 3–30 μL of this Cu^{2+} solution (1 mM) were transferred to each receptor solution (10 μM) to give 1 equiv. After mixing them for a few seconds, UV–vis spectra were taken at room temperature.

For Cys, **1** (7.8 mg, 0.003 mmol) was dissolved in DMSO (1 mL) and 10 μL of this solution (3 mM) were diluted with 2.99 mL of 10 mM bis-tris buffer/DMSO (1/1, v/v) to make the final concentration of 10 μM. $Cu(NO_3)_2\cdot 2.5H_2O$ (2.4 mg, 0.001 mmol) was dissolved in 10 mM bis-tris (1 mL) and 3 μL of this Cu^{2+} solution (10 mM) were transferred to each receptor solution (10 μM) to give 1 equiv. Then, L-cysteine (4.9 mg, 0.004 mmol) was dissolved in bis-tris (10 mM, 1 mL) and 3–30 μL of this Cys solution (40 mM) were transferred to **1** and Cu^{2+} solution (10 μM) to give 4 equiv. After mixing them for a few seconds, UV–vis spectra were taken at room temperature.

2.4. Job plot measurements

For Cu^{2+}, **1** (7.8 mg, 0.003 mmol) was dissolved in DMSO (1 mL). 12, 10.8, 9.6, 8.4, 7.2, 6.0, 4.8, 3.6, 2.4, 1.2 and 0 μL of the **1** solution were taken and transferred to vials. Each vial was diluted with bis-tris buffer/DMSO (7/3, v/v) to make a total volume of 2.988 mL. $Cu(NO_3)_2\cdot 2.5H_2O$ (7.3 mg, 0.003 mmol) was dissolved in bis-tris buffer (1 mL). 0, 1.2, 2.4, 3.6, 4.8, 6.0, 7.2, 8.4, 9.6, 10.8, and 12 μL of the $Cu(NO_3)_2$ solution were added to each diluted **1** solution. Each vial had a total volume of 3 mL. After reacting them for a few seconds, UV–vis spectra were taken at room temperature.

For Cys, **1** (7.8 mg, 0.003 mmol) was dissolved in DMSO (1 mL) and $Cu(NO_3)_2\cdot 2.5H_2O$ (7.3 mg, 0.003 mmol) was dissolved in 10 mM bis-tris (1 mL), respectively. The two solutions were mixed to make **1**-Cu^{2+} complex. 12, 10.8, 9.6, 8.4, 7.2, 6.0, 4.8, 3.6, 2.4, 1.2 and 0 μL of the **1**-Cu^{2+} complex solution were taken and transferred to vials. Each vial was diluted with bis-tris buffer/DMSO (1/1, v/v) to make a total volume of 2.988 mL. L-Cysteine (3.7 mg, 0.03 mmol) was dissolved in bis-tris buffer (10 mM, 1 mL). 0, 1.2, 2.4, 3.6, 4.8, 6.0, 7.2, 8.4, 9.6, 10.8, and 12 μL of the Cys solution were added to each diluted **1**-Cu^{2+} solution. Each vial had a total volume of 3 mL. After reacting them for a few seconds, UV–vis spectra were taken at room temperature.

2.5. Competition experiments

For Cu^{2+}, **1** (7.8 mg, 0.003 mmol) was dissolved in DMSO (1 mL) and 10 μL of this solution (3 mM) were diluted with 2.99 mL of 10 mM bis-tris buffer/DMSO (7/3, v/v) to make the final concentration of 10 μM. MNO_3 (M = Na, K, Ag, 0.03 mmol) or $M(NO_3)_2$ (M = Mn, Fe, Co, Ni, Cu, Zn, Cd, Hg, Mg, Ca, Pb, 0.03 mmol) or $M(NO_3)_3$ (M = Fe, Cr, Al, Ga, In, 0.03 mmol) were dissolved in 10 mM bis-tris (10 mL). 10 μL of each metal solution (3 mM) were taken and added to 3 mL of the solution of receptor **1** (10 μM) to give 1 equiv. of metal ions. Then, 10 μL of Cu^{2+} solution (3 mM) were added into the mixed solution of each metal ion and **1** to make 1 equiv. After mixing them for a few seconds, UV–vis spectra were taken at room temperature.

For Cys, **1** (7.8 mg, 0.003 mmol) was dissolved in DMSO (1 mL) and $Cu(NO_3)_2\cdot 2.5H_2O$ (7.3 mg, 0.003 mmol) was dissolved in 10 mM bis-tris (1 mL), respectively. The two solutions were mixed to make **1**-Cu^{2+} complex and 10 μL of this solution (3 mM) were diluted with 2.99 mL of 10 mM bis-tris buffer/DMSO (1/1, v/v) to make the final concentration of 10 μM. Various amino acids and peptide such as Gly, Ala, Ser, Thr, Val, Leu, Ile, Met, Pro, Phe, Trp, Asp, Glu, Asn, Gln, His, Lys, Arg and glutathione (GSH) (0.03 mmol) were dissolved in 10 mM bis-tris (10 mL). 10 μL of each amino acid and GSH solution (3 mM) were taken and added to 3 mL of the solution of Cys (10 μM) to give 1 equiv. of amino acids and GSH. Then, 10 μL of **1**-Cu^{2+} solution (3 mM) were added into the mixed solution of each amino acid or GSH and Cys to make 1 equiv. After mixing them for a few seconds, UV–vis spectra were taken at room temperature.

2.6. Determination of Cu^{2+} in water samples

UV–vis spectra measurement of water samples containing Cu^{2+} were carried by adding 20 μL of 3 mmol/L stock solution of **1** and 0.60 mL of 50 mmol/L bis-tris buffer stock solution to 2.38 mL sample solutions. After well mixed, the solutions were allowed to stand at 25 °C for 2 min before test.

2.7. Theoretical calculation methods

All DFT/TDDFT calculations based on the hybrid exchange-correlation functional B3LYP [34,35] were carried out using Gaussian 03 program [36]. The 6-31G** basis set [37,38] was used for the main group elements, whereas the Lanl2DZ effective core potential (ECP) [39–41] was employed for Cu. In vibrational frequency calculations, there is no imaginary frequency for the optimized geometries of **1** and **1**$^-$-Cu^{2+}, suggesting that these geometries represent local minima. For all calculations, the solvent